"AN OBSERVATIONAL CROSS-SECTIONAL STUDY TO COMPARATIVELY EVALUATE SERUM HOMOCYSTEINE LEVEL IN SUBJECTS WITH ORAL PRECANCEROUS LESION AND SQUAMOUS CELL CARCINOMA"

Dissertation submitted to

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In partial fulfillment of the requirement for the degree of

MASTER OF DENTAL SURGERY

In the specialty of

ORAL AND MAXILLOFACIAL PATHOLOGY AND ORAL MICROBIOLOGY

Deemed to be University
(Established u/s 3 of the UGC Act, 1956)

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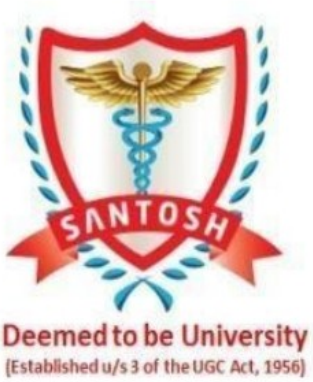

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LIST OF ABBREVIATIONS

PML	Pre-malignant Lesion
OSMF	Oral Submucous Fibrosis
OLP	Oral Lichen Planus
L	Leukoplakia
Hcy	Homocysteine
HNSCC	Head and Neck Squamous Cell Carcinoma
OSCC	Oral Squamous Cell Carcinoma
OPMD	Oral Potentially Malignant Disorders
OCM	One Carbon Metabolism
SAM	S-Adenosyl Methionine
MTHFR	5,10-methylenetetrahydrofolate reductase
HPV	Human Papilloma Virus
IHD	Ischaemic Heart Disease
MCI	Mild Cognitive Impairment
SPSS	Statistical Package for Social Sciences
SD	Standard Deviation

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INTRODUCTION

About 500,000 persons worldwide receive a head & neck cancer diagnosis each year, mostly squamous cell carcinoma of the larynx, pharynx, & oral cavity.[1] Overall, individuals with head & neck cancer have one of the lowest 5-year survival rates among the main tumour types, despite advancements in surgical procedures, chemotherapy, & radiation therapy.[2] Due to rise in chewing tobacco along with associated items like pan-masala, betel nut, & betel quid, oral cancer is always on the rise across the Indian subcontinent. Oral squamous cell carcinoma, or OSCC, develops as a result of a complicated process that includes genetic, epigenetic, & metabolic changes triggered by exposure to carcinogens. Oral submucous fibrosis (OSF), a potentially malignant disease (PMD), occurs before OSCC.[3]

Oral leukoplakia, oral submucous fibrosis (OSMF), & oral erythroplakia are most prevalent precancerous lesions of the oral cavity. In addition to these three conditions, other potentially malignant conditions for oral carcinoma include actinic cheilitis, a few other genetic diseases such xeroderma pigmentosum & Fanconi's anaemia, & immunodeficiency.[4] In a clinicopathological research, 70 out of 320 individuals in the lower or intermediate socioeconomic category had various oral premalignant lesions. The most prevalent premalignant condition was leukoplakia (20.65% of patients), although the percentages of patients with OSMF & lichen planus were comparable (0.62% of patients).[5]

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It is unclear what causes precancerous lesions of the oral mucosa. The course of development of possibly malignant oral disorders is significantly influenced by certain risk factors, including alcohol use, tobacco chewing, & tobacco smoking. Tobacco smoking could represent risk indicator for the oral leukoplakia, but tobacco chewing is a significant risk factor for OSMF, erythroplakia, & oral leukoplakia. Drinking alcohol may raise the risk of erythroplakia by 3 times, OSMF by 2 times, & oral leukoplakia by 1.5 times.[6]

The aetiology of OSCC is multifactorial. Alcohol & tobacco in all its forms are the most prevalent aetiological agents. Actinic radiation, trauma, tooth discomfort, human papillomavirus (HPV) infections, & dietary deficiencies such vitamin A, B, & C are other causes. The diagnosis & prognosis of cancer highly depends on the identification of the molecular markers/ biochemical markers which comprises the various metabolic processes connecting to the beginning & biological activity of specific tumours.[7]

According to several writers, homocysteine (Hcy) is a "tumour marker" for a number of malignancies, including colorectal, lung, cervical, breast, & many more. One biological marker of oral squamous cell carcinoma is homocysteine, an amino acid that is made from a different amino acid called methionine. Sulphur is found in cysteine, which is also involved in the body's metabolism of iron, zinc, & copper. It has antioxidant properties as well. Homocysteine levels rise if it is unable to pass through the methionine cycle or convert to cysteine.[8]

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An intermediate metabolite in the metabolism of methionine is homocysteine. It is broken down by two different pathways: the trans-sulphuration process, which first converts homocysteine to cysteine & subsequently to taurine, & the remethylation pathway, which promotes the regeneration of methionine. Because of its direct & indirect effects on the metabolism of methyl & sulphur groups, homocysteine has a crucial position in the metabolic pathway. A key factor in controlling chromosomal abnormalities, gene expression, & alterations is DNA methylation. The exact process by which high homocysteine levels may contribute to the development of cancer is yet unknown. Many in vitro studies have proposed different theories. Hyperproliferation, increased mutagenesis, abnormal apoptosis, aberrant methylation in the genome & loci, DNA damage, & DNA repair are some of these. The most commonly accepted theory is that the primary tumour suppressor genes have promoter hypermethylation. As a result, this amino acid could potentially be utilised to identify possibly cancerous conditions early on.[9]

Hcy is a new biomarker of folate deficiency & a sensitive indication of folate status. One-Carbon Metabolism (OCM), which includes purine & pyrimidine synthesis, amino acid metabolism, & the production of S-adenosylmethionine (SAM), a primary agent accountable for the DNA methylation, is dependant on the folate. Gene expression, chromosomal alterations, & chromosomal abnormalities are all significantly influenced by DNA methylation. OCM disruption can affect DNA synthesis, repair, & methylation, all of which can contribute to the development of cancer. [10]

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OCM requires folate as well as a number of vitamins & enzymes. In a process that requires vitamin B12, a one-carbon bond from folate is taken up by Hcy, creates methionine, whereas in the reaction which requires vitamin B6, Hcy gets broken down. Accumulation of Hcy may occur from impairment of either route. Low folate, vitamin B1, vitamin B6, & a frequent mutation (667C-T) in the methylenetetrahydrofolate reductase gene, that lowers enzyme activity, have all been linked to elevated Hcy levels. Serum Hcy may therefore be an integratory, sensitive biomarker of OCM disturbance that leads to the development of cancer.[11]

Few research have linked plasma homocysteine to PMDs of the oral cavity along with additional locations, while few publications have found higher homocysteine levels. This study, which assesses the plasma homocysteine levels in OSCC, is only the third of its sort.

Therefore, the purpose of this current research was to measure the plasma homocysteine levels within the control groups, patients with oral precancerous lesions, & with OSCC; to compare the levels across age groups; & to measure the levels as OSCC & oral precancerous lesions progressed. The feasibility of use of plasma homocysteine as a biomarker was another goal of this investigation.

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AIM & OBJECTIVES

AIM:

To calculate the levels of serum Homocysteine & serum folate in oral precancerous lesion & OSCC patients as well as the healthy subjects.

OBJECTIVES:

1. To determine the levels of serum Homocysteine & serum folate in oral precancerous lesion, OSCC patients & healthy subjects.

2. To assess the levels of serum Homocysteine & serum folate in oral precancerous lesion, OSCC patients & healthy subjects.

3. To measure the clinical utility of serum Hcy as a potential tumor marker for the oral precancerous lesion & OSCC.

4. To compare both group of serum Homocysteine & the serum folate in oral precancerous lesion, OSCC patients & healthy subjects.

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REVIEW OF LITERATURE

Weinstein SJ, Ziegler RG, Selhub J. (2001)[12] investigated the connection between invasive cervical cancer & blood homocysteine, an effective biomarker for folate deficiency & issues with one-carbon metabolism. High-performance liquid chromatography was used to detect serum homocysteine, & an enzyme-linked immunosorbent assay was utilized to measure exposure to HPV type 16, the most common carcinogenic strain of the virus. Women in the top 3 homocysteine quartiles (> 6.31 micromol/L) had a significantly higher risk of invasive cervical cancer; multivariate-adjusted odds ratios varied from 2.4 -3.2. There was a noticeable & important trend. The odds ratios were comparable when cases were matched solely with HPV-16 seropositive controls. Therefore, it was determined that the risk of cervical cancer that is invasive was highly & significantly predicted by serum homocysteine. This correlation may be due to genetic variants that impact one-carbon metabolism, folate, or deficiencies in B12 and/or B6.

Wu LL, Wu JT. (2002)[13] Despite not receiving antifolate medication, the study discovered that cancer patients had higher levels of circulating total homocysteine (tHcy), & this shift was correlated with the concentration of tumour markers in individuals receiving therapy. The concentration of tHcy & the tumour marker would both rise concurrently while the tumour cell grew, however just the Hcy concentration would decrease as the tumour cell died. Hyperhomocysteinemia has been linked to a number of biochemical alterations, such as oxidative stress, abnormal DNA methylation, folate insufficiency, & the synthesis of homocysteine

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thiolactone, which accounted for the eventual carcinogenesis caused by high homocysteine. Hence, hyperhomocysteinemia as a carcinogenesis risk factor & tHcy as a precise tumour marker for tracking the cancer patients during therapy.

Almadori G, Bussu F, Galli J et al (2002)[14] examined the levels of two metabolites, homocysteine & folate, in the blood of 42 patients with head & neck squamous cell carcinoma against control groups. Carcinoma patients and smokers had mean folate levels of 5.8 +/- 2.1 ng/mL, 9.1 +/- 2.7 ng/mL, and 9.7 +/- 2.2 ng/mL, respectively. The two groups' differences were statistically significant. The mean total homocysteine levels in all three groups varied statistically significantly: 8.3 +/- 2.8 microM for smokers, 7.8 +/- 2.5 microM for non-smokers, & 10.4 +/- 5.3 microM among carcinoma patients. Consequently, it was demonstrated that changes in levels in the blood of homocysteine and folate may be caused by cancer growth and the resulting metabolic alterations, or they may precede and promote tumour progression. In patients who have precancerous lesions as well as those with treated HNSCC who are susceptible to loco-regional recurrence & second primary tumours, folate may have a role as a new chemopreventive drug if hypofolatemia is a risk indicator for neck & head carcinogenesis.

Weinstein SJ, Gridley G, Harty LC et al. (2002)[15] carried out a population-based, case-control research in Puerto Rico to ascertain the associations between oral cancer risk, methylenetetrahydrofolate reductase C677T polymorphism genotype, blood homocysteine levels, & folate & methionine consumption. Despite the fact that a higher intake of folate was linked to a lower risk of oral

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cancer, this finding was mostly attributable to fruit consumption, since other dietary sources of folate did not clearly correlate. There was no correlation between the incidence of oral cancer and serum homocysteine levels or methionine intake. Patients who carried the MTHFR C677T homozygous variant genotype had a non-significantly lower risk, and risk patterns seemed to vary by degree of consumption of alcohol, smoking, methionine, and folate, even if the ability to find significant correlations in subgroups of these variables was limited. Consequently, it was concluded that there is no unique risk of mouth cancer associated with folate; rather, advice for preventing this disease should highlight the importance of a healthy diet and a high fruit intake.

Sun CF, Haven TR et al. (2002)[16] has out a research to examine the production of tumour markers & the amount of homocysteine produced by tumour & normal cell lines. The amounts of homocysteine produced by tumour cells were much greater. However, when Hcy concentration was conveyed per the same number of cells, a significantly smaller difference between normal & tumour cell lines was seen. For the first seven days of the cell culture, the concentration of the tumour marker & the Hcy increased in tandem. Tumour markers increased during the seventh day of the culture, when the cells began to die, whereas Hcy & cell counts stabilised. Therefore, it was determined that, in cancer patients who were not receiving anti-neoplastic medication, the blood concentration of Hcy varied in circulation in tandem with the tumour marker. The fast multiplication of tumour cells may be the reason of the elevated tHcy concentration, which alone indicates the quantity of living cells. To track tumour activity, serum Hcy could be a helpful tumour marker.

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Chandalia M, Abate N, Cabo-Chan AV et al. (2003)[17] aimed to ascertain if Asian Indians in the United States, during the period of folate fortification, had higher plasma homocysteine levels than Caucasians, & whether or not low vitamin B-12 or insulin resistance may be the cause of any interethnic variations in plasma homocysteine levels. It was discovered that Asian Indians had lower plasma concentrations of B-6 & much greater plasma homocysteine than Caucasians. Both ethnic groups' plasma folate levels were comparable & comparatively high. Compared to Caucasians, Asian Indians had much lower plasma vitamin B-12 levels. For all vitamin B-12 levels, Asian Indians reported noticeably higher homocysteine levels. As a result, it was shown that whereas Asian Indians in the US had normal plasma folate levels, their plasma homocysteine levels are significantly elevated. Lower insulin sensitivity & vitamin B-12 plasma concentrations may be involved in this discovery, however they only partially account for Asian Indians' ethnically associated hyperhomocysteinemia.

Eleftheriadou A, Chalastras T, Ferekidou E et al. (2006)[18] investigated the relationship between the clinical stage & the blood levels of homocysteine & folate in patients with squamous cell carcinoma of head & neck. The serum levels of homocysteine & folate were assessed in 150 healthy individuals (77 smokers & 73 nonsmokers) & 150 participants with histologically confirmed SCCHN prior to therapy. According to the study, the existence of SCCHN was positively correlated with both hyperhomocysteinemia & hypofolatemia. Many healthy smokers were found to have excessive homocysteine levels & folate deficiencies. Therefore, it was determined that methionine cycle metabolic abnormalities are

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verified indicators of risk for SCCHN, & that dietary supplements including folate may help with chemoprevention & posttreatment care for SCCHN patients.

Kalita J, Misra UK, Srivastava AK et al (2007)[19] examined the degree of homocysteine in healthy people and compared them to plasma B12, folic acid, and dietary habits. In a cross-sectional study, 200 healthy volunteers had their demographic, clinical, and laboratory characteristics assessed. Compared to nonvegetarians, vegetarians exhibited a significantly higher plasma Hcy level. Nonetheless, folic acid and plasma B12 levels were significantly lower among vegetarians. Multivariate analysis revealed that hyperhomocysteinemia was only associated that had low plasma B12 vitamin levels. Therefore, it was determined that vegetarians had greater Hcy levels than nonvegetarians, which were linked to lower plasma B12 levels.

Gorgulu O, Selcuk T, Ozdemir S et al. (2010)[20] examined the pathophysiology of laryngeal squamous cell carcinoma (LSCC) by assessing the levels of blood homocysteine, folate, & vitamin B(12) in 60 consecutive untreated LSCC patients & 60 controls (30 smokers & 30 non-smokers). Vitamin B12 levels in LSCC patients were considerably lower than those in the smoker & non-smoking control groups. Additionally, compared to the smoker & non-smoking control groups, the folate levels in LSCC patients were considerably lower. The homocysteine levels in these three groups did not differ significantly. Therefore, it was determined that metabolic changes in vitamin B(12) & folate levels, especially hypofolataemia, may be linked to the onset of LSCC, however more investigation is needed to clarify their functions with certainty.

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Bais PS, Chauhan P, Mohan S. (2013)[21] conducted a research to assess the biological marker of blood homocysteine levels in patients with oral submucous fibrosis. The study comprised 50 individuals with clinically & histologically confirmed oral submucous fibrosis who were not receiving therapy. Of the patients, 54% were in stage II, 36% were in stage III, & 10% were in stage IV. The total amount of homocysteine was 24.1 µmol/lit. Both male & female patients had comparable homocysteine levels. Patients in stage IV had greater homocysteine levels than those in stages III & II. The change was not statistically significant, though. Gender & clinical stage did not significantly affect homocysteine levels. To determine the true effect of serum homocysteine, longer follow-ups & a longitudinal investigation including a greater number of patients having OSMF are required.

Kamdi SP, Palkar P. (2013)[22] We out a study to find out how common hyperhomocysteinemia is among India's healthy upper socioeconomic level. The levels of total homocysteine (tHcy) were measured in 1243 healthy Indian physicians of various ages (906 men & 337 women). Hyperhomocysteinemia was found in 92.85% of males & 81.60% of women. Men were more likely than women to have hyperhomocysteinemia, with a mean tHcy concentration that was noticeably greater. Therefore, it was determined that the current subgroup of the population had an exceptionally high rate of hyperhomocysteinemia, which may indicate a future risk for a number of diseases.

Narang D, Shishodiya S, Sur J et al. (2014)[23] carried done research to determine whether serum homocysteine may be utilised to diagnose O.S.M.F. Fifty

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individuals with a clinical & pathological diagnosis of O.S.M.F. who were not receiving therapy were the subjects of the study. Every patient in their research, regardless of age or gender, had an elevated blood homocysteine level. When comparing the homocysteine levels between clinical phases & pathological grading, there was no significant statistical correlation. In order to determine the extent of the illness and as an prognosticator for disease management, it was demonstrated that there was a significant increase in blood homocysteine in OSMF, suggesting that chronic inflammation in OSMF results in hyperhomocysteinemia.

Zhang D, Wen X, Wu W et al. (2015)[24] investigated the relationship between blood levels of homocysteine, folate, as well as vitamin B12 & 5,10-methylenetetrahydrofolate reductase polymorphism & overall cancer risk. There was no heterogeneity by cancer type or ethnicity, as well as elevated Hcy but low folate levels were associated with an overall increased risk of cancer. Vitamin B12 levels only showed a negative correlation with malignancies of the gastrointestinal and urinary systems, along with those in patients from Asia and the Middle East. Additionally, the MTHFR C677T, A1298C, and G1793A polymorphisms were associated with elevated blood levels of Hcy, and especially deficits in folate and vitamin B12. However, MTHFR C677T homogeneity/wild type (TT/CC) was the only polymorphism that showed a substantial correlation with overall cancer risk. Consequently, it was demonstrated that a higher blood Hcy level and a folate deficiency are associated with an increased overall risk of cancer.

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Jaganath SS, Kaveri H, Okade A. (2016)[25] evaluated plasma homocysteine levels in OSF, OSCC, & healthy controls in order to assess its plausibility as a possible biomarker in these disorders. Compared to healthy controls, OSF & OSCC patients had higher plasma homocysteine levels. In contrast, OSCC patients had lower levels than OSF patients. A changed plasma homocysteine level, however statistically insignificant, is essential to the pathophysiology of many disorders because it causes oxidative DNA damage, which starts the carcinogenesis process. It is a measure of vitamin B12 & folate levels, & taking supplements of these vitamins may help cure OSCC, stop the advancement of OSF, & fight hyperhomocysteinemia. Early identification, management, & treatment effectiveness monitoring would be aided by coordinated efforts.

Erugula SR, Kandukuri MK, Danappanavar PM et al. (2016)[26] We out a research to compare the concentrations of serum homocysteine and serum folate in OSCC patients, smokers, and healthy individuals, as well as to assess the clinical use of serum Hcy as a potential tumour marker in OSCC. Of the 60 individuals in the study, 15 were non-OSCC smokers and 30 were diagnosed as OSCC patients. The control group consisted of 15 healthy individuals who did not smoke. Blood folate levels in OSCC patients were 5.34 ng/mL on average, 7.68 ng/mL in the smokers group, and 10.99 ng/mL in the placebo group. The average blood folate levels of the three study groups varied considerably from one another. That there is an intriguing correlation between blood Hcy & folate levels in OSCC, which may serve as a biochemical "tumour marker" & shed light on the disease's genesis & course.

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Ara R, Goodman A, Pervin S et al. (2017)[27] measured the concentrations of homocysteine within blood of the women having cervix SCC & healthy controls, where they were evaluated for the demographic & socioeconomic characteristics of 50 women with aggressive squamous cell carcinoma & 50 healthy controls. Of the instances of cervical cancer, 14% had high homocysteine levels & 82% had levels between 4.5 & 15 µmol/L. On the other hand, 98% of the control group exhibited homocysteine levels between 4.5 and 15 µmol/L, while 2% had levels below that range. Furthermore, the average homocysteine level of cervical cancer patients and controls differed statistically significantly. Consequently, it was shown that the levels of homocysteine in women with cervical cancer vary. Dietary deficiencies in fruits and vegetables are associated with homocysteine, an indicator for or co-factor associated with the malignant growth of the cervical squamous epithelium. However, increasing public awareness of the potential advantages of vitamin B-rich fresh fruits & vegetables in reducing homocysteine levels and avoiding cancer is crucial.

Gorji HAM, Heydari AM, Rokni FR et al. (2017)[28] evaluated each patient's blood levels of folic acid, vitamin B12, & homocysteine. Descriptive statistics & an independent t-test were used to examine the data. Confounding factors were controlled for using logistic regression. Abnormally elevated blood homocysteine levels were found in 36.66% of patients with cutaneous-oral lichen planus & 30% of individuals with psoriasis. Although the psoriatic group's mean & maximum blood folic acid levels were greater than those of the cutaneous-oral lichen planus group, the difference was not statistically significant. Although there were no appreciable differences between the 2 groups, blood homocysteine levels were

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greater in those with psoriasis & cutaneous-oral lichen planus, 2 chronic inflammatory skin conditions, than in healthy control patients. Therefore, it was determined that before starting treatment, more research is advised about the course of action for people with psoriasis & lichen planus.

Kilic N, Dagli N, Aydin S et al. (2017)[29] compared the levels of ghrelin, obestatin, homocysteine, vitamin B12, & folate in the blood & saliva of patients with ischaemic heart disease. The ELISA technique was used to measure the levels of obestatin, Hcy, & acylated & desacylated ghrelin. While acylated & desacylated ghrelin levels in the saliva had been considerably lower than those in the serum, obestatin, acylated ghrelin, & desacylated ghrelin levels in the saliva were found to be greater than those in the control group's serum. Patients with IHD had greater levels of obestatin. IHD patients had substantially reduced amounts of vitamin B12 & folate in their saliva & blood compared to those in the other group. As a result, it was determined that individuals with ischaemic heart disease had higher blood ghrelin levels & lower serum obestatin levels.

Chiang CP, Yu-Fong Chang J et al. (2018)[30] evaluated the homocysteine levels of 320 OLP patients in a research. Twenty-eight.1%, 8.4%, 1.6%, 26.3%, 21.3%, & 24.4% of 320 OLP patients had specific blood antinuclear, anti-smooth muscle, anti-mitochondrial, gastric parietal cell, thyroglobulin, & thyroid microsomal autoantibodies, respectively. Additionally, we found that 352 OLP patients had excessively high blood homocysteine levels & deficits in haemoglobin, iron, vitamin B12, & folic acid in 21.9%, 13.6%, 7.1%, 0.3%, & 14.8% of cases, respectively. Therefore, before beginning treatment for OLP patients, it is crucial to check their blood autoantibody, haematinic, & homocysteine levels. The

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preferred medication for treating OLP is corticosteroids as it is an immunologically driven condition.

Damanik J, Mayza A, Rachman A et al. (2019)[31] has out a research to determine the relationship between middle-aged T2DM populations' blood homocysteine levels & cognitive impairment. Forty-seven (48.5%) of the patients had moderate cognitive impairment. The domain with the greatest impairment was delayed recollection (94.8%). Serum Hcy levels in the MCI & non-MCI groups did not differ significantly on average. After controlling for confounding factors, the final logistic regression model revealed no correlation between serum Hcy & cognitive performance. To validate this conclusion, more research with a bigger sample size & somewhat older T2DM patients should be done.

Vanjani MV, Phulari RGS, Rathore R. (2019)[32] conducted a research to compare & link the blood Hcy & vitamin B12 levels in individuals with OSMF. The trial group included sixty patients. Using chemiluminescence immunoassay, serum Hcy & vitamin B12 were estimated. Compared to healthy controls, OSMF had higher Hcy levels, which were statistically significant. Conversely, despite the fact that healthy controls had greater vitamin B12 levels, the difference was not statistically significant. Hcy levels & OSMF severity did not correlate, although there was a strong link between Hcy & vitamin B12 within both groups. Thus, it was shown that hyperhomocysteinemia, which can also occur in situations of vitamin B12 deficiency & several systemic illnesses, is caused by persistent inflammation in OSMF. Consequently, serum Hcy may serve as a biomarker for OSMF.

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Hasan T, Arora R, Bansal AK et al. (2019)[33] carried out research to assess the homocysteine levels of cancer patients. Their research findings suggest that Hcy may be employed as a tumour marker. Despite not receiving anti-folate medication, they discovered that cancer patients had higher levels of circulating total homocysteine. The much elevated levels of circulating tHcy were caused in part by the tumour cells' fast growth. Only the Hcy concentration would decrease in response to tumour cell death, whereas both the tHcy & tumour marker concentrations would rise concurrently during tumour cell growth. Hyperhomocysteinemia has been linked to a number of biochemical alterations, such as oxidative stress, abnormal DNA methylation, folate insufficiency, & the synthesis of homocysteine thiolactone, which explains why high homocysteine ultimately causes carcinogenesis. It was determined that hyperhomocysteinemia is an indicator of risk for carcinogenesis & that tHcy can be utilised as more precise tumour marker for the tracking of cancer patients during the therapy.

Yang Q, Lu Y, Deng Y et al. (2020)[34] has out a research to demonstrate the relationship between changes in renal function & blood fasting homocysteine levels in hypertension patients. Only in men, & regardless of confounders, were homocysteine levels linked to blood urea nitrogen (BUN) & serum creatinine levels at coefficients of 2.04 & 0.07, respectively. Serum uric acid levels in male patients were inversely correlated with haemoglobin A1c (HbA1c) levels, high density lipoprotein cholesterol levels, & the occurrence of stroke, but body mass index was favourably correlated. On the other hand, only LVEF showed a favourable correlation with female uric acid levels. Therefore, it was determined

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that in male patients having hypertension, homocysteine levels are an independent risk factor linked to blood creatinine & BUN levels.

Debnath L, Chowdhury AW, Alam I et al. (2020)[35] carried out a research in a tertiary care hospital in Bangladesh to investigate the function of these CAD markers in patients recently diagnosed acute coronary syndrome who were type II diabetes & those who were not. Serum homocysteine levels were substantially higher in group II than in group I. Similarly, group I's hsCRP was 26.84±30.30 mg/L, but group II's was higher at 37.48±37.99 mg/L. Patients in group II, both male & female, had greater levels of Hcy & hs-CRP. Thus, serum Hcy & hs-CRP were shown to be considerably greater in non-diabetic individuals with ACS than in those having type 2 diabetes. There is more room for in-depth research in the future because this link can be demographic or ethnicity specific.

Azzini E, Ruggeri S, Polito A. (2020)[36] performed a review with an emphasis on the nutritional management of increased Hcy levels. It also examined the function of homocysteine in various demographic groups, particularly in risk circumstances, & its significance as a marker & etiological feature for the illnesses in these age groups. Hcy levels during pregnancy were examined in connection with the elevated risk of unfavourable pregnancy outcomes, including intrauterine growth restriction, hypertension, small size for gestational age at delivery, & repeated abortions. Therefore, it was determined that the success rate of vitamin supplementation in lowering Hcy levels & improving pathological conditions, particularly in older patients with overt pathologies, has not been

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conclusively established by clinical trials. This suggests that other dietary as well as non-dietary factors might be involved in high Hcy levels.

Wu YH, Lin PY, Yang JH et al. (2021)[37] carried out a study to determine whether patients with oral leukoplakia who were positive for the carcinoembryonic antigen or squamous cell carcinoma antigen had significantly greater serum homocysteine levels & a significantly greater incidence of haematinic deficiencies & hyperhomocysteinemia compared to patients who were CEA-negative or SCC-Ag-negative or healthy control subjects. They discovered that compared to 184 healthy control subjects, 85 CEA-positive as well as 25 SCC-Ag-positive OL patients had significantly greater rates of serum folic acid deficiency & hyperhomocysteinemia, as well as significantly lower mean serum folic acid & homocysteine levels.

Furthermore, compared to the 159 SCC-Ag-negative OL patients, the mean blood homocysteine level of the 25 SCC-Ag-positive OL participants was noticeably greater. Compared to 99 CEA-negative OL patients, 85 of the CEA-positive OL patients exhibited a higher mean blood homocysteine level & a greater incidence of hyperhomocysteinemia patients, respectively. Therefore, it was determined that compared to CEA-negative or SCC-Ag-negative OL, patients with CEA-positive / SCC-Ag-positive OL often have higher mean blood homocysteine levels & a greater incidence of hyperhomocysteinemia.

Palaskar S, Joshi K, Mukkanwar R et al. (2022)[38] investigated the serum Hcy levels in individuals with oral squamous cell cancer (OSCC). The clinical usefulness of serum Hcy as a possible tumour marker for OSCC sufferers was also

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evaluated in this investigation. Serum Hcy levels in OSCC patients & healthy people were examined & contrasted. Patients with OSCC had increased serum Hcy levels. Therefore, it was determined that blood Hcy levels might be used as biological indicators for OSCC patient diagnosis & prognosis.

Bahramian A, Dabaghi Tabriz F, Katebi K et al. (2023)[39] have out research to measure the amount of homocysteine in the serum & saliva of patients with SCC & compare the results with those of healthy people. 21 individuals with oral SCC & 21 healthy participants were examined in this case-control analytical investigation. An HCY ELISA Kit was used to measure the homocysteine levels in saliva & serum samples. Serum homocysteine levels were 2.01 ± 2.11 in the control group & 3.71 ± 2.5 in the sick group. The case group's salivary homocysteine levels remained 3.12 ± 1.66, whereas the control group's were 2.93 ± 1.71 (P = 0.782). Therefore, it was determined that blood homocysteine levels might be a useful marker for the diagnosis of oral SCC; more study is required to determine if salivary homocysteine levels can be used as a diagnostic indication.

Samadi FM, Sivakumar N, Sonam M et al. (2024)[40] Salivary trace element concentrations may therefore be diagnostically significant in the early assessment of OSCC and OPMDs, it was determined. Elevated concentrations of these components may serve as a prognostic indicator and a marker of the course of the disease. Salivary trace element levels were shown to be diagnostically relevant for evaluating OPMDs and OSCC at an early stage. Increased levels of these substances might be a sign of the disease's progression and a prognostic indication.

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Sandhya G, Monisha S, Singh S et al. (2024)[41] investigated the relationship between elevated homocysteine & changes in brain volume & cognition in a group of 1296 middle-aged & older persons. The subjects were divided into low & high Hcy groups according to their median homocysteine levels (16.89 µmol/L). The Generalised Linear Model (GLM) discovered a significant correlation between high Hcy & vocabulary task after controlling for age, gender, years of schooling, vitamin B12, folate, & dyslipidaemia. There were also notable correlations seen between high Hcy & cerebral white matter volume. Therefore, it was determined that individuals with high Hcy levels had smaller cerebral white matter volumes & performed worse on cognitive tests pertaining to the language domain. This suggests that homocysteine may have a significant effect on both the anatomy & function of the brain.

Vahed MM, Bahramian A, Katebi K et al. (2024)[42] Through a systematic review & subsequent meta-analyses, their study sought to evaluate the blood levels of homocysteine & folate in patients with HNSCC with healthy controls. Because the chosen publications varied in their discussion of smoking behaviours, several meta-analyses were conducted to determine if the blood levels of homocysteine & folate in patients with HNSCC differed from those of healthy controls. Serum folate levels were much lower in HNSCC patients than in the control groups, regardless of smoking status. Likewise, the sick groups' blood homocysteine levels were noticeably greater than those of the control groups. Therefore, it was shown that blood homocysteine & folate levels were associated with HNSCC, suggesting that they might be used as biomarkers for the early identification of HNSCC.

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MATERIALS & METHOD

SOURCE OF DATA

A sample of 140 participants in the Department of Oral & Maxillofacial Pathology at Santosh Dental College in Ghaziabad, Uttar Pradesh, participated in the current cross-sectional study. Participants in the research had to be at least eighteen years old. Subjects were chosen at random, regardless of gender. The Institution's Ethical Committee granted ethical approval for the study's execution. The research was carried out from June 2023 to June 2024.

SAMPLE SIZE:

The research had 140 participants in all. Of them, 70 will be categorised as the control group (Group III), 35 as oral precancerous lesions (Group I), & 35 as OSCC (Group II).

SAMPLE SIZE CALCULATION:

The sample size for the current research was 140 patients.

It is calculated by this formula**: -**$n = \frac{2\,(Z\alpha + Z\beta)^2\,\sigma^2}{\delta^2}$

Whereas**:** $Z\alpha$ = level of statistical significance (2.326 for 99% level of confidence),

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$Z\beta$ = desired power (1.28 for Power= 0.80) σ^2 = standard deviation (1.52 + 1.67)/2 = 1.59 δ^2 = difference of 2 group mean (X_2-X_1) = 9.60 - 8.60 =1.00

INCLUSION CRITERIA

1. Either gender, age above 18 years old.

2. All oral precancerous lesions & Stage 1 well differentiated & poorly differentiated OSCC diagnosed histopathologically.

3. Healthy people without a history of systemic disorders, medication, smoking, or tobacco chewing were included in the control group.

EXCLUSION CRITERIA

1. People with conditions including diabetes mellitus, alcoholism, Alzheimer's disease, osteoporosis, Parkinson's disease, coronary artery disease, peripheral vascular disease, the development of deep vein etc. that are known to change the blood homocysteine profile.

2. Individuals on drugs capable of altering Homocysteine levels.

3. Individuals below 18 years.

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ARMAMENTARIUM

1. Ice box

2.. Sterile bottles

3. Folate Estimation Kit

4. Syringe

5. Anticoagulants

6. Centrifuge machine

7. Fluorescence polarization immunoassay kit

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METHODLOGY

Of the 140 participants in the trial, 35 were assigned to the oral precancerous lesion group (Group I), 35 to the OSCC group (Group II), & 70 to the control group (Group III).

Every patient & healthy volunteer with appropriate renal function who did not take a supplement of folate in the year before the trial began. All patients & healthy individuals provided their informed permission in accordance with a college Ethical Committee policy.

Subjects for the study Group I & Group II were clinically examined for precancerous lesion & oral malignancy & then subjected to histopathological confirmation before including them in the study. The rest of the subjects were kept in control Group III. Blood samples were taken in the morning, & all research participants were instructed to fast overnight to prevent any food influences. After 45 minutes for the blood to coagulate, it was centrifuged for 10 minutes at 2500 rpm. An Eppendorf tube was filled with serum that had been extracted from blood samples. After being kept at -20°C, serum samples were transported to a lab for further processing.

As directed by the manufacturer, the Architect immunoassay optical system was used to detect the Serum Folate levels utilising the Chemiluminescent Microparticle Immunoassay (CMIA) technology, also known as Chemiflex.

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A Snibe Maglumi 800 Fluorescence Polarisation immunoassay Kit Analyser, which use chemiluminescent immunoassay technology to determine blood homocysteine quantitatively, was used to test homocysteine.

Group I, Group II, & Group III values were acquired, & an intergroup comparison was performed. The Proforma was filled out with all of the data at once, & a master chart was created. After being tallied, the findings were forwarded for statistical analysis.

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STATISTICAL ANALYSIS

SPSS statistical software, version 23.0 (IBM, USA), was used for the statistical analysis. For every question, the data were organised in frequencies (percentages), & the mean percentage was determined. Frequency & % were included in the descriptive statistics. The mean & standard deviation were used to measure a few of the parameters. For the current investigation, the significance threshold was set at 5%. The Chi Square test was used to compare the ordinal variable between groups.

FORMULA USED FOR THE ANALYSIS

The Arithmetic Mean

The most widely used measure of central tendency is the arithmetic mean, which is usually referred as the mean, & is calculated using

$$\bar{X} = \frac{\sum_{i=1}^{n} X_i}{n}$$

The Standard Deviation

The standard deviation is the positive square root of the variance, calculated with :

$$SD = \sqrt{\frac{\sum X_i^2 - \frac{(\sum X_i)^2}{n}}{n-1}}$$ where n = no. of observations

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Chi Square Test

A statistical test called chi-square is frequently used to compare data that has been observed with data that would be expected based on a certain hypothesis. An analyst may question how accurately a statistical model captures the data when they try to fit it to observable data. This test is frequently used to examine the relationship between variables in two-way tables, where the actual data is compared to the expected model of independence. The chi-square test result often has the following form:

$$X^2 = \sum \frac{(\text{observed - expected})^2}{\text{expected}}.$$

One Way ANOVA

The formula applied for the one-way **ANOVA** *F*-test is

$$F = \frac{\text{between-group variability}}{\text{within-group variability}}.$$

The in between-group variability is calculated using

$$\sum_{i=1}^{K} n_i (\bar{Y}_{i\cdot} - \bar{Y})^2 / (K-1)$$

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where Y_i denotes the sample mean in the i^{th} group, n_i are the no. of observations in the i^{th} group, Y denotes the overall mean of the data, & K stands for the number of groups. The within-group variability is

$$\sum_{i=1}^{K}\sum_{j=1}^{n_i}(Y_{ij}-\bar{Y}_{i\cdot})^2/(N-K),$$

where Y_{ij} is the j^{th} observation in the i^{th} out of K groups & N is the sample size.

Independent t-test

The Independent t Test is most frequently employed when the test statistic corresponds to a normal distribution & may be used to ascertain whether two sets of data differ substantially from one another. When two distinct sets of independently distributed samples are produced, one from each of both of the populations under comparison, the t-test for independent samples is employed.

$$t=\frac{\overline{X}_1-\overline{X}_2}{\sqrt{\left(\frac{(N_1-1)s_1^2+(N_2-1)s_2^2}{N_1+N_2-2}\right)\left(\frac{1}{N_1}+\frac{1}{N_2}\right)}}$$

Where X1 represents the mean of the first group, X2 symbolises the mean of the second group

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LEVEL OF SIGNIFICANCE (P-VALUE)

- The highest likelihood that a true null hypothesis will be rejected.
- The Level of Significance of the Test is the highest probability at which we would be ready to take a risk while testing a certain hypothesis. ≥ 0.05 is the non-significant P-value. P-value < 0.05 indicates significance, P-value < 0.01 indicates high significance, & P-value < 0.001 indicates very high significance.

When the p-value was less than 0.05 ($p<0.05$), it was considered significant, & a 95% confidence interval was used.

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Figure 1: Precancerous lesion – Leukoplakia

Figure 2: Precancerous lesion – Oral Lichen Planus

Figure 3: Precancerous condition – Oral Submucous Fibrosis

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Figure 4: Oral Squamous Cell Carcinoma

Figure 5: Armamentarium for Biopsy Procedure

Figure 6: Armamentarium for withdrawal of blood

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Figure 7: Centrifuge for extraction of serum

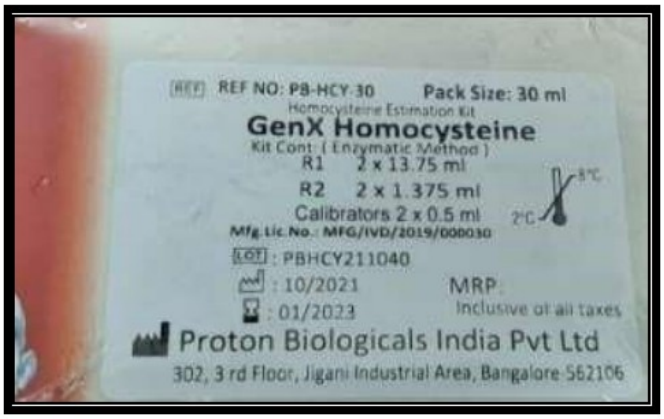

Figure 8: Fluorescence Polarization Immunoassay Kit for Serum Homocysteine Level Estimation

Figure 9: CMIA kit for Serum Folate Level Estimation

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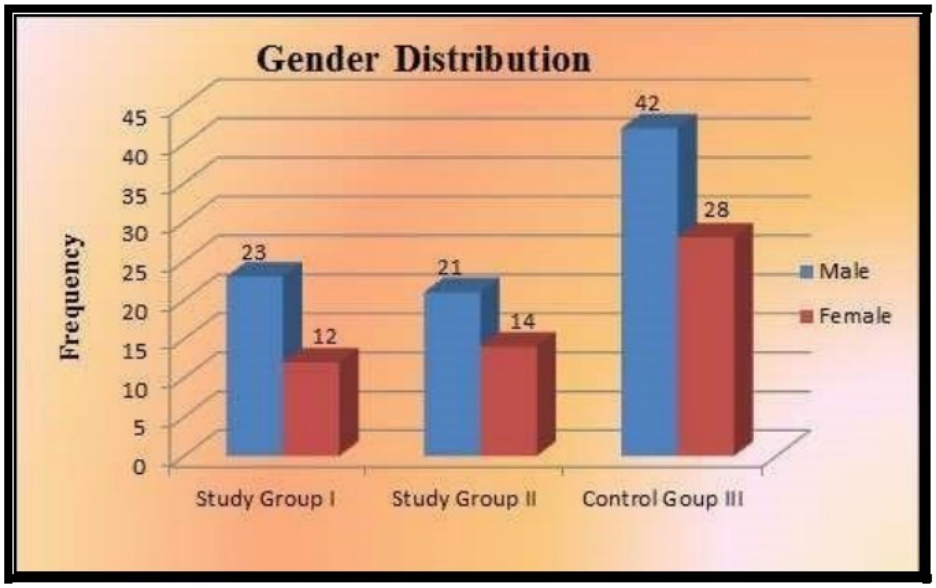
RESULTS

In the current research were 140 subjects, comprising of 35 subjects in the Study Group I- Precancerous Lesion (23 males & 12 females), 35 subjects in the Study Group II Oral Squamous Cell Carcinoma (21 males & 14 females) & 70 healthy subjects in the Control Group III (42 males & 28 females). **(Table 1, Graph 1)**

Table 1: Gender Distribution

Group	Male	Female
Study Group I	23	12
Study Group II	21	14
Control Group III	42	28
Total	86	54



Graph 1: Gender Distribution

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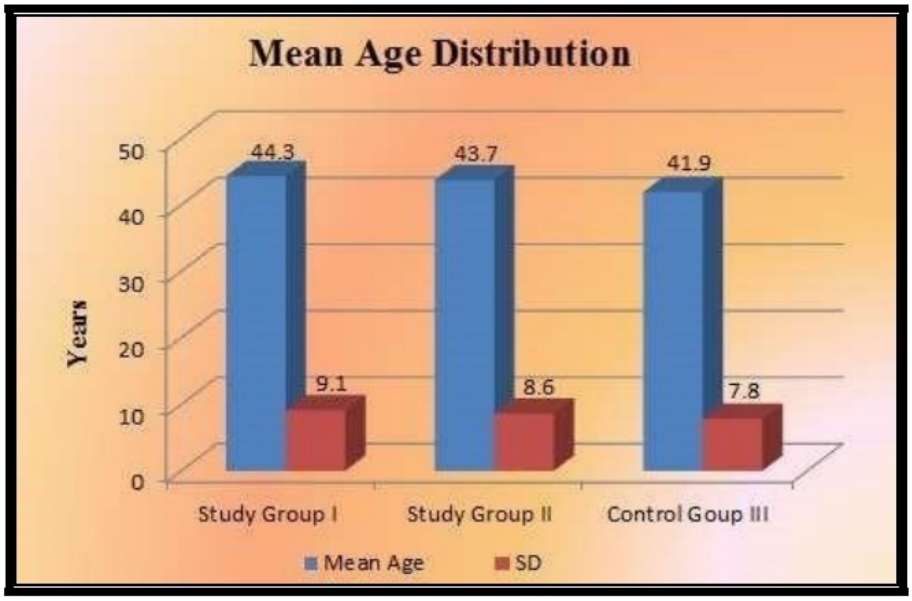
Mean Age Distribution: The individuals in Study Group I had a mean age of 44.3 ± 9.1 years, with a range of 22 to 61 years. The individuals in Study Group II were between the ages of 24 & 58, with an average age of 43.7 ± 8.6 years. The patients in Control Group III were between the ages of 21 & 54, with an average age of 41.9 ± 7.8 years. **(Table 2, Graph 2)**

Table 2: Mean Age Frequency Distribution

Group	Mean Age (years)	SD (years)
Study Group I	44.3	9.1
Study Group II	43.7	8.6
Control Group III	41.9	7.8



Graph 2: Mean Age Distribution

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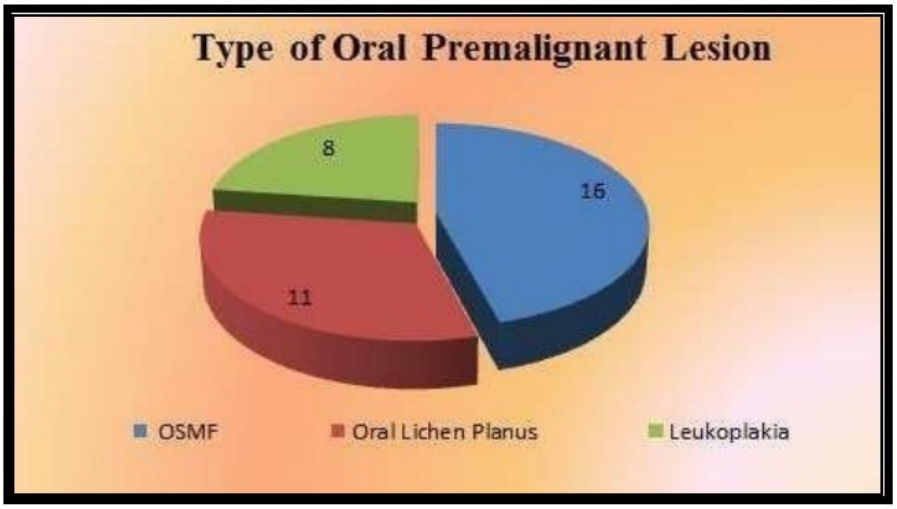
TYPES OF ORAL PREMALIGNANT LESION IN GROUP I

Group I comprised of 35 oral premalignant lesion subjects. 16 (45.7%) subjects had OSMF, 11(31.5%) subjects had Oral Lichen Planus & 8 (22.8%) subjects had leukoplakia. **(Table 3, Graph 3)**

Table 3: Type of Oral Premalignant Lesion

Lesion	Frequency	Percentage
OSMF	16	45.7%
Oral Lichen Planus	11	31.5%
Leukoplakia	8	22.8%
Total	35	100%



Graph 3: Type of Oral Premalignant Lesion

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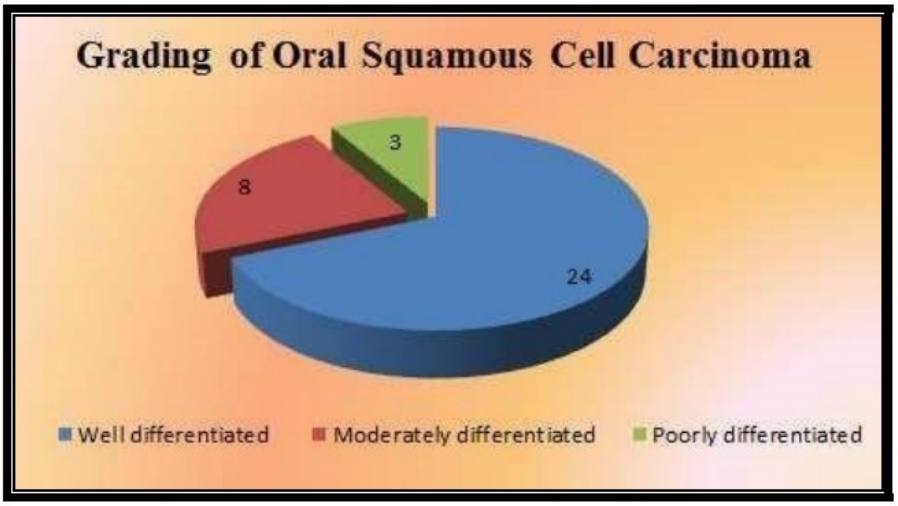
GRADING OF ORAL SQUAMOUS CELL CARCINOMA IN GROUP II

Group II comprised of 35 oral squamous cell carcinoma subjects. 24 (68.6%) subjects had OSMF, 8 (22.8%) subjects had Oral Lichen Planus & 3 (8.6%) subjects had leukoplakia. **(Table 4, Graph 4)**

Table 4: Frequency distribution of grading of the Oral Squamous Cell Carcinoma

Grading	Frequency	Percentage
Well differentiated	24	68.6%
Moderately differentiated	8	22.8
Poorly differentiated	3	8.6
Total	35	100%



Graph 4: Grading of Oral Squamous Cell Carcinoma

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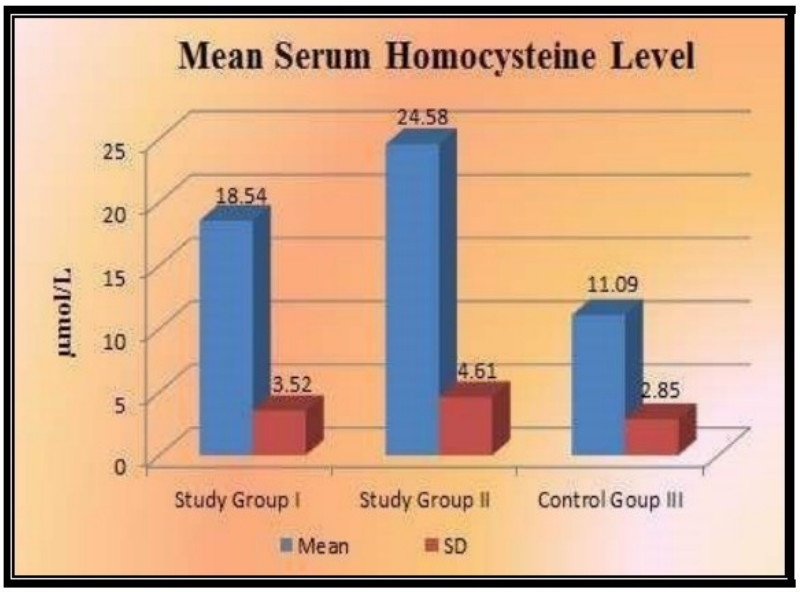
COMPARISON OF THE MEAN SERUM HOMOCYSTEINE LEVEL

Study Group I – oral premalignant lesion subjects had mean serum Homocysteine level 18.54±3.52 µmol/L. Study Group II – oral squamous cell carcinoma subjects had mean serum Homocysteine level 24.58±4.61 µmol/L & Study Group III – healthy subjects had mean serum Homocysteine level 11.09±2.85 µmol/L. The statistical significance of the difference between the groups was significantly high. **(Table 5 & 6, Graph 5)**

Table 5: Mean Serum Homocysteine Level

Group	Mean Hcy Level (µmol/L)	SD (µmol/L)
Study Group I	18.54	3.52
Study Group II	24.58	4.61
Control Goup III	11.09	2.85



Graph 5: Mean Serum Homocysteine Level

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Table 6: One Way ANOVA Test for Serum Homocysteine Level

	SERUM HOMOCYSTEINE LEVEL					
	N	Mean (µmol/L)	Std. Deviation	Std. Error	F-value	p-value
Group I	35	18.54	3.52	1.990	19.506	0.01**
Group II	35	24.58	4.61	2.819		
Group III	70	11.09	2.85	1.703		
One-way ANOVA test			**** Highly significant difference**			

COMPARISON OF MEAN SERUM FOLATE LEVEL

Study Group I – oral premalignant lesion subjects had mean serum folate level 5.47±1.72 ng/mL. Study Group II – oral squamous cell carcinoma subjects had mean serum folate level 3.67±1.24 ng/mL & Study Group III – healthy subjects had mean serum folate level 8.91±2.98 ng/mL. The differences within the groups were highly statistically significant (Table 7 & 8, Graph 6)

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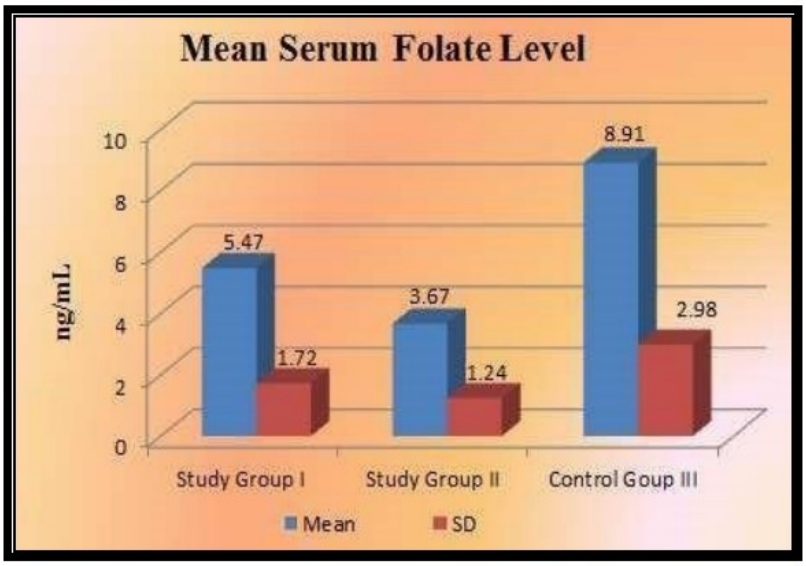
Table 7: Mean Serum Folate Level

Group	Mean Folate Level (ng/mL)	SD (ng/mL)
Study Group I	5.47	1.72
Study Group II	3.67	1.24
Control Group III	8.91	2.98



Graph 6: Mean Serum Folate Level

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Table 8: One Way ANOVA Test for Serum Folate Level

	SERUM FOLATE LEVEL					
	N	Mean (ng/mL)	Std. Deviation	Std. Error	F-value	p-value
Group I	35	5.47	1.72	1.427	12.506	0.01**
Group II	35	3.67	1.24	2.182		
Group III	70	8.91	2.98	1.934		
One-way ANOVA test			**** Highly significant difference**			

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DISCUSSION

As the sixth most frequent cancer globally, squamous cell carcinoma of the head & neck (HNSCC) is the most prevalent head & neck cancer. [43] Oral cancer, which makes up 2–4% of all malignancies, is the most prevalent kind of HNSCC. It has a high death rate & is most frequently identified as oral squamous cell carcinoma. [44]

Oral cancer is on a perpetual rise in the Indian subcontinent, owing to an increase in chewing tobacco & allied products like betel nut, betel quid, pan-masala etc. Oral squamous cell carcinoma is a multi-step process of epigenetic, genetic, & metabolic alterations brought on by exposure to carcinogens leads to the development of cancer. It arises from the pharynx, larynx, & oral cavity's mucosal epithelium. N early ninety percent of all oral cancers are OSCC, which has a 40–50% five-year survival rate. One of the worst health issues in the world & the leading cause of mortality, its prevalence has been rising in emerging nations in the twenty-first century. High rates of morbidity & mortality seem to be caused by the incapacity to diagnose patients early & provide the right therapy.[45]

Oral cancer is mostly caused by tobacco smoking in all its forms, along with excessive alcohol use. Other causes include high UV & solar radiation levels, vitamin A as well as C deficiency, HPV infections, trauma, & dental irritation. Oral cancer may be caused by genetic damage resulting from exposure to environmental factors. Gene mutations have been found in several chromosomes in OSCC. Molecular anomalies in oral carcinogenesis are mostly caused by genes

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related to cell cycle regulation & control, which offer the mutant population of cells a growth advantage.[46]

Carcinogenesis may be caused by or contribute to the growth of tumours & is also associated with metabolic alterations. Therefore, examining metabolic anomalies might be a useful strategy for comprehending the biochemistry of tumours & could lead to the discovery of novel therapeutic targets. [47] One important metabolic mechanism, the methionine cycle, has been linked to carcinogenesis. The metabolism of methionine in the production of different proteins affects a number of metabolic functions, such as the production of nutrients needed for the best possible operation of the skeletal, neurological, & cardiovascular systems. [51] Two routes are involved in the metabolism of homocysteine (Hcy), which is produced as a by-product of methionine metabolism.[48]

First, homocysteine is converted to cysteine and then taurine by the trans-sulphuration process; second, methionine is restored via the re-methylation route. Homocysteine, an intermediate metabolite, is essentially located at a critical metabolic crossroads that affects all methyl and sulphate group metabolism within the body, both directly and indirectly. [49] Elevated blood homocysteine levels can be caused by a variety of internal and external factors. [50]

Genetic variation is the most significant internal component, whereas dietary composition is the most significant external factor. Despite not being directly present in the food, homocysteine is linked to both vitamin B12 & folate. [51] Vitamin B12 & folate function as co-enzymes & cofactors in homocysteine, respectively, & are crucial in the conversion of homocysteine into methionine.[52]

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Homocysteine is a methionine cycle intermediate cross-linking molecule that has a direct or indirect impact on all bodily metabolisms connected to methyl & sulphur groups. A developing biomarker of folate deficiency, homocysteine is a reliable measure of folate status. [53] One-Carbon Metabolism, which includes purine & pyrimidine synthesis, amino acid metabolism, & the production of S-adenosylmethionine, the primary agent needed for the methylation of DNA, depends on folate. Gene expression, chromosomal alterations, & chromosomal abnormalities are all significantly influenced by DNA methylation. DNA synthesis, repair, & methylation can all be hampered by interruption of one carbon metabolism, which may encourage the development of cancer.[54]

Folate, along with other vitamins & enzymes, is necessary for single-carbon metabolism. In a reaction which requires B12 vitamin, a single-carbon group from folate is taken up by Hcy for producing the methionine, whereas within a reaction that requires vitamin B6, Hcy gets broken down. [55] Hcy may build up as a result of impairment of either mechanism. Low folate, vitamin B1, vitamin B6, & a common mutation within the methylenetetrahydrofolate reductase gene, whereby lowers enzyme activity, have all been linked to elevated Hcy levels. Serum Hcy may therefore be an integratory, sensitive biomarker of a disturbance in 1-carbon metabolism that leads to the development of cancer.[56]

OSCC is preceded by oral lesions such as OSMF, oral lichen planus, & leukoplakia, which are classified as potentially malignant disorders (PMD). PMDs may be classified as having a high risk of malignant transformation by biomarkers such as plasma free amino acids, which might also help with early identification

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& treatment. [57] Since amino acids & their derivatives reflect protein metabolism, issues with food absorption, & help uncover metabolic disturbances that occur throughout the pathological processes brought on by PMDs & cancer, they can be helpful molecular or disease indicators. [58] Plasma homocysteine, a non-essential amino acid, is considered a valuable indicator of vitamin status due to its strong correlation with vitamin B12, folic acid, & vitamin B6. Plasma homocysteine has only been linked in a small number of studies to PMDs of the oral cavity along with other locations, with some findings indicating higher homocysteine levels. [59] According to the results, patients with SCC & oral premalignant lesions had much higher blood homocysteine levels than healthy individuals. The absence of vitamin B12 & folic acid, which function as cofactors as well as co-substrates in the metabolism of homocysteine, may be one of the causes of the rise in homocysteine levels in the patient group.[60]

Group I, Group II, & Group III had an average serum homocysteine levels ranging from 18.54±3.52 µmol/L, 24.58±4.61 µmol/L, & 11.09±2.85 µmol/L, respectively, in this investigation. Group I, Group II, & Group III had mean blood folate levels of 5.47±1.72 ng/mL, 3.67±1.24 ng/mL, & 8.97±2.98 ng/mL, respectively, in our research.

The results of our investigation aligned with a research conducted by Palaskar et al.[38], who found a significant difference in homocysteine levels between cancer patients and both smoking and non-smoking controls.

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Eleftheriadou et al.[18] found that HNSCC patients had substantially higher blood homocysteine levels & lower serum folate levels than both the smoking group & healthy patients. These findings align with those of the current investigation.

When comparing HNSCC patients to smokers & healthy persons, Almadori et al.[14] found that the former had considerably higher blood concentrations of homocysteine & lower levels of folate. According to the authors, tobacco smokers may experience chromosomal damage in their buccal mucosa cells as a result of folate & B12 shortages. This study's findings were consistent with ours. Our findings were consistent with a research by Jaganath et al.[25] that assessed plasma homocysteine levels in individuals suffering from OSMF & oral SCC & found that these patients had greater plasma homocysteine levels than healthy participants.

The findings of our investigation were comparable to those of Zhang et al. investigations.[24] According to research by , Kamdi et al.[22], & Bais et al.[21] Narang et al.[23], & Ergula et al.[26], plasma homocysteine levels rose in all OSMF patients, irrespective of age or sex.

Our study's findings conflicted with those of Weinstein et al.[12] & Damanik et al.[22], who both observed no discernible difference between blood homocysteine & serum folate in individuals with OSCC & oral premalignant lesions. According to a research by Gorgulu et al.[20] there were none of the discernible difference between SCC & control groups. The fact that patients in the current research had oral SCC whereas those in Gorgulu et al.'s research had laryngeal SCC may be the cause of the discrepancy in the outcomes.[20]

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Overall, it was shown that participants with oral cancer & premalignant lesions had significantly higher blood homocysteine levels & lower serum folate levels than healthy subjects. Subjects from a single demographic group & a very limited sample size were the study's limitations. Therefore, to confirm our findings for a wider population, a bigger sample size comprising participants from other fields should be included.

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CONCLUSION

This study investigated the blood levels of homocysteine and folate in patients having OSCC, oral premalignant lesions, and healthy individuals using the chemiluminescence immunoassay method. Increased blood levels of homocysteine were seen within the patients with OSCC as well as oral premalignant lesions compared to healthy individuals.

It was concluded that serum homocysteine level was a sensitive marker to screen for the subclinical deficiencies of micronutrients like folate & Vitamin B12. Since the deficiency of vitamin B12 & folates are shown to be causative of OSF & OSCC, homocysteine could be used as a plausible biomarker in detecting OSF & OSCC at the early stages of the disease. Since hypofolatemia leads to hyperhomocysteinemia, folic acid, Vitamin B6 & B12 supplementation can be used as chemopreventive agents, which act as a primary mode of prevention & reduce the risk of cancer development in high risk patients. It is likely to enhance the prognosis & decrease the formation of second primary tumours & loco-regional reappearance of the main illness in OSCC cases that have already received treatment. The findings provide credence to the idea that homocysteine may be employed as a tumour biomarker in cases with OSCC. However, to validate this idea, more study needs be conducted with a bigger sample size & to rule out the impact of dietary variables.

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ANNEXURES

ANNEXURE I

DEPARTMENT OF ORAL PATHOLOGY AND MICROBIOLOGY

SANTOSH DENTAL COLLEGE AND HOSPITAL

PROFORMA

"AN OBSERVATIONAL CROSS-SECTIONAL STUDY TO COMPARATIVELY EVALUATE SERUM HOMOCYSTEINE LEVEL IN SUBJECTS WITH ORAL PRECANCEROUS LESION AND SQUAMOUS CELL CARCINOMA"

Serial. No.	
Name	
Age	
Sex	
Premalignant Lesion	Group I-Oral Premalignant/ Group II-OSCC/ Group III-Healthy
Grading of OSCC	Well Differentiated/ Moderately Differentiated/ Poorly Differentiated
Serum Homocysteine Level	…….µmol/L
Serum Folate Level	…….ng/mL

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ANNEXURE II

DEPARTMENT OF ORAL PATHOLOGY AND MICROBIOLOGY
SANTOSH DENTAL COLLEGE AND HOSPITAL

INFORMED CONSENT

I_____as a legally responsible citizen hereby give my consent for the survey study titled **"AN OBSERVATIONAL CROSS-SECTIONAL STUDY TO COMPARATIVELY EVALUATE SERUM HOMOCYSTEINE LEVEL IN SUBJECTS WITH ORAL PRECANCEROUS LESION AND SQUAMOUS CELL CARCINOMA"** being conducted by **DR. RUPINDER MINHAS**, postgraduate student, under the guidance of **Dr. NEERAJ GROVER,** Professor & Head, Department of Oral Pathology and Microbiology, Santosh Dental College and Hospital Dental Sciences & Research. I have been promised anonymity of the information provided by me. I hereby voluntarily, unconditionally give my consent, without any fear or pressure in mentally sound and conscious state to let myself to participate in this study.

Witness Participant's Signature

Date:

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ANNEXURE III

MASTER CHART FOR STUDY GROUP I – PRECANCEROUS LESION

Case No	Age (years)	Gender	Precancerous Lesion	Serum Homocysteine level (µmol/L)	Serum Folate level (ng/mL)
1	33	M	OSMF	18.53	6.39
2	48	M	L	19.39	2.72
3	40	F	OLP	16.93	7.42
4	30	F	OSMF	18.53	6.39
5	42	M	OLP	21.39	3.75
6	52	M	OSMF	12.93	7.42
7	38	M	OSMF	18.53	3.39
8	43	M	L	29.39	2.46
9	40	M	OLP	16.93	7.42
10	35	M	OSMF	18.53	6.39
11	61	F	OSMF	19.39	4.02
12	46	M	OLP	16.98	6.42
13	36	F	OSMF	18.53	5.39
14	33	M	L	19.39	4.82
15	35	M	OLP	14.93	7.42
16	47	M	OSMF	18.53	6.39
17	58	F	OSMF	19.39	3.92
18	49	M	L	16.93	7.42
19	48	M	OSMF	18.53	6.39
20	22	M	L	19.39	3.85
21	51	F	OLP	16.93	7.42
22	54	M	OSMF	18.53	6.39
23	52	M	OSMF	19.39	4.24
24	43	F	OLP	16.93	7.42
25	58	F	OSMF	22.55	5.39
26	52	M	L	19.39	3.98
27	22	F	OLP	16.93	7.42
28	49	F	L	18.58	3.39

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29	43	M	OSMF	19.39	6.01
30	46	F	OLP	26.93	4.42
31	42	M	OSMF	18.57	6.39
32	61	M	L	19.39	3.22
33	38	F	OLP	16.93	5.42
34	43	M	OSMF	20.53	6.39
35	40	M	OLP	19.35	4.27

Key: L-Leukoplakia, OLP-Oral Lichen Planus, OSMF-Oral Submucous Fibrosis, M-Male, F-Female

MASTER CHART FOR STUDY GROUP II - OSCC

Case No	Age (years)	Gender	OSCC	Serum Homocysteine level (µmol/L)	Serum Folate level (ng/mL)
1	33	F	WD	28.53	2.39
2	48	M	WD	19.39	2.72
3	40	F	PD	26.93	3.42
4	30	M	WD	18.53	6.39
5	42	F	WD	21.39	3.75
6	52	M	MD	32.93	2.42
7	38	F	WD	28.53	3.39
8	43	M	WD	29.39	2.46
9	40	F	MD	16.93	3.42
10	35	M	WD	18.53	6.39
11	48	F	WD	32.39	4.02
12	46	M	MD	26.98	2.42
13	36	F	WD	18.53	5.39
14	33	M	WD	29.39	4.82
15	35	M	WD	24.93	2.42
16	47	M	WD	38.53	6.39
17	58	F	WD	19.39	3.92
18	52	M	MD	26.93	4.42
19	48	M	PD	28.53	2.39
20	32	M	MD	29.39	3.85

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21	51	M	WD	16.93	5.42
22	54	F	WD	28.53	2.39
23	52	F	WD	29.39	4.24
24	24	M	WD	16.93	2.42
25	58	F	MD	22.55	2.39
26	52	M	WD	19.39	3.98
27	64	F	PD	20.93	3.42
28	24	F	WD	18.58	3.39
29	43	M	MD	19.39	2.01
30	46	F	WD	26.93	4.42
31	42	M	WD	38.57	2.39
32	52	M	WD	29.39	3.22
33	38	M	MD	16.93	5.42
34	43	M	WD	20.53	2.39
35	40	M	WD	19.35	4.27

M-Male, F-Female, OSCC-Oral Squamous Cell Carcinoma, WD-Well differentiated, MD-Moderately differentiated, PD-Poorly differentiated

MASTER CHART FOR CONTROL GROUP III - HEALTHY

Case No	Age (years)	Gender	Serum Homocysteine level (µmol/L)	Serum Folate level (ng/mL)
1	21	M	10.66	8.39
2	48	F	13.26	8.72
3	40	M	12.34	7.42
4	30	M	11.53	10.39
5	54	F	10.39	9.75
6	52	M	12.93	9.42
7	38	M	9.83	10.39
8	43	F	8.39	7.46
9	40	M	11.93	9.42
10	35	M	8.53	8.39
11	48	F	9.39	10.02

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12	46	M	10.98	9.42
13	36	F	11.53	9.39
14	33	M	12.39	8.82
15	35	F	11.93	8.42
16	47	M	10.53	6.39
17	52	F	10.39	9.92
18	49	M	6.95	7.42
19	48	M	10.53	8.39
20	32	M	13.39	9.85
21	48	F	11.93	10.42
22	46	M	11.53	6.39
23	36	F	12.39	14.24
24	33	M	8.93	7.42
25	35	M	12.55	5.39
26	47	M	10.66	8.39
27	50	F	13.26	8.72
28	48	F	12.34	7.42
29	46	M	11.53	10.39
30	36	F	10.39	9.75
31	42	M	12.93	9.42
32	52	M	9.83	10.39
33	38	F	8.39	7.46
34	43	M	10.66	8.39
35	40	M	13.26	8.72
36	35	M	12.34	7.42
37	48	F	11.53	10.39
38	46	M	10.39	9.75
39	36	F	12.93	9.42
40	33	M	9.83	10.39
41	35	M	8.39	7.46
42	47	M	10.66	8.39
43	52	F	13.26	8.72
44	49	M	13.39	9.85
45	48	M	11.93	10.42

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46	50	M	10.39	9.92
47	51	F	6.95	7.42
48	42	F	10.53	8.39
49	52	F	13.39	9.85
50	38	M	11.93	10.42
51	43	M	11.53	6.39
52	42	F	10.39	9.92
53	52	F	6.95	7.42
54	38	M	10.53	8.39
55	43	M	13.39	9.85
56	42	F	11.93	10.42
57	52	F	11.53	6.39
58	38	M	10.39	9.92
59	43	M	6.95	7.42
60	42	F	10.53	8.39
61	52	F	13.39	9.85
62	38	M	11.93	10.42
63	43	M	11.53	6.39
64	42	F	10.39	9.92
65	52	M	6.95	7.42
66	57	M	13.39	9.85
67	44	F	11.93	10.42
68	49	M	11.53	6.39
69	42	F	13.39	9.85
70	52	M	11.93	10.42

Key: M-Male, F-Female

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सन्तोष डेन्टल कॉलेज

प्रताप विहार, गाजियाबाद

सहमति पत्र

1. मैं .....पुत्र/पत्री.....निवासी..... ..... अपने ऊपर किये जाने वाले निरीक्षण/ परीक्षण, चिकित्सीय सलाह/पद्धति की पूर्ण सहमति देता/देती हुं।

2. मुझे चिकित्सा प्रणाली और चिकित्सा के उपरान्त होने वाले संभावित नतीजों के बारे में दंत चिकित्सक के द्वारा अच्छी तरह समझाया गया है।

3. मुझे चिकित्सा के दौरान अथवा उसके उपरांत होने वाली संभावित/ अप्रत्याशित हानियों, इलाज के समय उपयुक्त होने वाली दवा व उसके प्रयोग के बारे में भी अच्छी तरह बताया गया है।

4. रोगी के द्वारा चिकित्सा के बीच में ही छोड़कर जाने अथवा चिकित्सा के उपरान्त परिणाम के उम्मीद के अनुसार न आने पर, चिकित्सक की जिम्मेदारी नहीं होगी।

5. मैं पुनः अपनी सहमति देता हूं कि इस रिकार्ड को भविष्य में चिकित्सीय शोध, प्रशिक्षण एवं चिकित्सा प्रणाली के उत्थान के लिये प्रयोग किया जा सकता है।

तारीखः

रोगी के हस्ताक्षर

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Dr Rupinder Minhas

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F. No. SU/2023/2480[26] Dated: 26.09.2023

Subject: Grant of Ethical Clearance for Thesis Protocol Topic "An observational cross-sectional study to comparatively evaluate serum homocysteine level in healthy subjects, subjects with oral precancerous lesion and squamous cell carcinoma", submitted by **Dr. RUPINDER MINHAS,** MDS student of Batch 2022-2023, Department of **ORAL PATHOLOGY & MICROBIOLOGY**.

*****

With reference to his/her request for grant of Ethical Clearance for the Thesis Protocol entitled "An observational cross-sectional study to comparatively evaluate serum homocysteine level in healthy subjects, subjects with oral precancerous lesion and squamous cell carcinoma", **Dr. RUPINDER MINHAS** is informed that the Thesis Protocol submitted by him/her was considered by the **Screening Committee** of the Santosh Dental College & Hospital in its meetings held on 07.02.2023 & 08.02.2023. The recommendations of the **Screening Committee** were considered in detail by the Institutional **Ethics Committee** in its meeting held on **23.09.2023** and the same was **approved** by the **Ethics Committee**.

He /She is informed accordingly for further necessary action.

[DR. NARESH SHARMA]
REGISTRAR

SANTOSH "DEEMED TO BE UNIVERSITY" REGISTRAR GHAZIABAD, (NCR) U.P.

Dr. RUPINDER MINHAS
MDS student of batch 2022-2023
Department of ORAL PATHOLOGY & MICROBIOLOGY
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Copy to:
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5. The Director - IQAC
6. **Dr. Neeraj Grover** (Guide)
7. HOD of **ORAL PATHOLOGY & MICROBIOLOGY**

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